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Original article

Identification of oleosins as major allergens in sesame seed allergic patients

Background: The prevalence of sesame allergy is increasing in European countries. Cases of severe allergy lack any evidence of specific immunoglobulin (Ig)Es by prick tests and CAPSystem-FEIA. The reasons for this negativity are unknown.
Methods: In 32 patients displaying immediate symptoms such as anaphylactic shock, asthma, urticaria, angioedema, sesame allergy was diagnosed by double-blind placebo-controlled food challenge (DBPCFC) or convincing clinical history. However, 10 patients had negative prick tests and CapSystem-FEIA. The specificity of IgEs was further investigated by enzyme-linked immunosorbent assay (ELISA), isoelectrofocalisation (IEF)-blotting, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) blotting using total sesame extracts and purified fraction of oil bodies. Monospecific rabbit antibodies directed to two oleosin isoforms (15 and 17 kDa) were used.
Results: By ELISA, white sesame seed extract allowed the detection of higher levels of IgE than brown sesame extract. In all sera, numerous bands binding IgEs were detected by IEF or SDS-PAGE. In reducing conditions, two bands (15–17 kDa), could be separated from 2S albumin. Oleosins, present in oil bodies fractions, were recognized by IgEs from all sera.
Conclusion: Oleosins are major allergens of sesame seeds and may be relevant to severe anaphylaxis. Falsely negative prick tests could be due to the lack of oleosins in presently available extracts, or to the fact that epitopes might be buried in the inner molecule. Detection tests currently used to identify sesame allergens based on sesame vicillins or other storage proteins could be insufficient for the detection of sesame seed contamination. Oleosins have been named Ses i 4 (17 kDa) and Ses i 5 (15 kDa), in accordance with the IUIS Nomenclature Committee.

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Food allergy to sesame has been observed in children and in adults in different countries including Israel, Japan, and Europe (1–5). Anaphylaxis is often severe (6–8). The prevalence is increasing in European countries and could represent 2–4% of total food allergies (9, 10). Sesame is among the 12 allergens requiring labelling on food products (11).

The major allergen of sesame seeds has already been described. Ses i 1 (9 kDa) is a member of 2S albumin family (12) and recognized by all the patients studied ($n = 10$). More recently, Beyer et al. (13) identified two additional sesame allergens: Ses i 2 (7 kDa) and Ses i 3 (45 kDa), which are a subunit of 2S albumin and a 7S vicilin-like globulin, respectively. A 14 kDa protein belonging to the 2S albumin family was recognized by 22 of the 24 sera used (13). Numerous other allergens have been observed by two-dimensional electrophoresis followed by immunoblotting (14).

Cases of anaphylaxis have been reported despite negative prick tests and absence of specific immunoglobulin (Ig)Es (15, 16), where the authors suggested that the anaphylaxis was IgG-mediated. Other data have confirmed a potent immunogenicity of sesame seeds eliciting a polyisotypic response, supporting this assumption (17).

The aim of this study was to thoroughly investigate 32 sera from patients allergic to sesame, part of them displaying no evidence of allergen-specific sensitization by prick tests and CAP-FEIA. Enzyme-linked immunosorbent (ELISA) tests, immunoblotting after isoelectrofocalisation (IEF), and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electro-

Abbreviations: IEF, isoelectrofocalisation; pNPP, *p*-nitrophenyl-phosphate; CNBr, cyanogen bromide; NC, nitrocellulose; SPT, skin prick test.

phoresis were carried out with a white sesame (WS) seed extract and purified oil bodies containing the two isoforms of oleosins.

Specific IgEs to the oleosin fraction were detected in almost all sera from subjects allergic to sesame with positive or negative prick tests and CAP-FEIA. These results indicate that oleosins represent a new class of sesame major allergens.

Materials and methods

Patients

Patients ($n = 32$) including 15 children and 17 adults were selected and determined to exhibit sesame allergy as evidenced by double-blind placebo-controlled food challenge (DBPCFC), labial test or clinical history (Table 1). Patients were ranged according to the tests performed: 23 patients were positive after food challenge (labial test or DBPCFC), four patients did not react to 965 mg and 7 g, and five patients were not tested. Among the 23 patients, six patients were negative by skin prick tests (SPT) to three varieties of sesame seeds and to the commercial extract, as well as with the CAP-FEIA, which was performed twice for each case. One of them (patient 1) experienced a prelethal shock after the consumption of an industrial dish containing an artificial flavoring in a matrix of Mexican sesame oil. Allergy to sesame was very likely in cases 26 and 27, because of anaphylactic episodes related to successive ingestions of sesame seeds or of food products that may have contained sesame seeds or sesame oil.

Skin prick tests

The SPT were carried out with three varieties of natural sesame seeds (white, brown, and black), crushed in saline according to the technique of prick-in-prick (18) and compared with a commercial extract (Allerbio, Varennes en Argonne, France). The positive and negative controls were codein phosphate 9% and saline buffer, respectively. Criteria for positivity was a wheal diameter equal to 75% of the positive control wheal.

CAP-FEIA

The patients' specific IgE levels were determined by using the CAPSystem IgE to sesame (f10 Pharmacia, Uppsala, Sweden). Levels of specific IgE of > 0.35 kU/l (≥classe 1) were considered positive.

Food challenge

The DBPCFC was performed as previously described (19). Briefly, the progression increased up to a cumulative dose (CD) of 965 mg. The vehicle used was a stewed apple and the placebo material was crushed brown-dried toast.

The DBPCFC using cold-pressed sesame oil was tested in nine patients, successively using 1, 5, and 10 ml volumes. The placebo was paraffin oil (mineral oil) (20).

Sesame seed extracts

Brown and white sesame (WS) seeds were ground and extracted (1 : 5, w/v) overnight in sodium bicarbonate 4‰ at 4°C. After centrifugation, extracts were filtered on a 0.2 μm filter. Protein content was measured according to the Bradford method with serum albumin as standard. Protein concentrations were 5.5 mg/ml for brown sesame (BS) and 2.95 mg/ml for WS extract.

Oil bodies were extracted from sesame seeds and subjected to further purification using the protocol developed by Tzen et al. (21) including two-layer flotation by centrifugation, detergent washing, ionic elution, treatment of chaotropic agent (urea 9 M), and integrity testing with hexane.

Direct ELISA

Specific IgE were assessed by direct ELISA using white and BS seed extracts. The ELISA plates (Immulon 2; Thermo Lab systems, Franklin, MA, USA) were coated with white or BS seed extracts (10 μg/ml in carbonate buffer, pH 9.6) at 4°C overnight. Plates were washed with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBS-T) and blocked with 0.5% (w/v) gelatin in PBS-T at 37°C for 1 h. Plates were incubated at 37°C for 2 h with 100 μl per well of patient sera diluted 1 : 5 in blocking buffer. After three washes, an alkaline phosphatase-coupled affinity purified goat antihuman IgE antibody (KPL, Gaithesburg, MD, USA) diluted 1 : 1000 in blocking buffer was used to detect bound IgE. After 2-h incubation at 37°C, plates were washed three times and the color reaction was started with *p*-nitrophenylphosphate (pNPP) in diethanolamin buffer (pH 9.5). The absorbance was determined at 410 nm (A410 nm). All determinations were carried out in duplicates. Positive absorbance was defined as the mean background absorbance ± 3 SD. A *Dactylis glomerata* pollen extract and a grass pollen-sensitized human serum (CAP-FEIA g3 500 kU/l) were used as the standard.

Isoelectric focusing

Isoelectric focusing (IEF) was performed on 1.5% agarose gel with 2.6% ampholytes 3-10 (Serva, Heidelberg, Germany). Samples of 160 μg/cm were applied on the anodic part of the gel. After focusing, the gel was submitted to a 10 min pressure blot onto cyanogen bromide (CNBr)-activated nitrocellulose (NC; 22).

SDS-PAGE

The SDS-PAGE was performed on a 12% acrylamide gel with a 6% stacking gel in a Tris-Tricine buffer (23). Extracts were diluted in 20 mM Tris-HCl, pH 6.8, containing 2% (w/v) SDS and bromphenol blue. In reducing conditions, 1% (w/v) dithiothreitol (DTT) was added to the sample buffer and samples were incubated for 10 min at 95°C before application on the gel. Proteins of 20 μg/cm were applied for blotting and 2 μg per lane for silver staining. Electrophoresis was performed at 15°C at 40 mA for 1 h. After electrophoresis, the gel was electroblotted onto CNBr-activated NC or silver-stained according to Rabilloud et al. (24).

Immunodetection

The IEF or SDS-PAGE strips were blocked in 2% polyvinylpyrrolidone (PVP) in Tris-buffered saline (TBS) containing 0.1% (v/v) Tween 20 (TBS-T) for 1 h. Blots were then incubated at room temperature overnight with individual serum diluted 1 : 5 in blocking buffer. After three washes in TBS-T, blots were incubated with alkaline phosphatase-labeled antihuman IgE in blocking buffer (1/2000) for 6 h. After three washes in TBS-T, IgE-binding was revealed using NBT/BCIP (KPL). For the detection of oleosins, NC strips were incubated with polyclonal

Table 1. Clinical characteristics of 32 patients with sesame allergy

				Prick test		IgE CAP-RAST	DBPCFC/LT to sesame seeds (S) and/or oil (O)	
ID	Sex	Age (years)	Symptoms	CE	Varieties	(kU/l)	DBPCFC	LT (grade)
1	M	18	RAS	Neg	B: neg/W: neg/b: neg	<0.35	O (15 ml): rash, asthma/S (100 mg): urticaria	
2	F	63	RAS to 162 mg	ND	B: neg/W: neg/b: neg	<0.35	S (965 mg): systemic reaction	
3	F	9	nf AS	Neg	B: neg/W: neg/B: neg	<0.35	O (8 ml): late onset erythema	
4	M	44	AS	Neg	B: neg/W: neg/B: neg	<0.35	O (5 ml): AS/S (200 mg): flush, facial erythema	
5	M	54	AS	Neg	B: neg/W: neg/b: neg	<0.35	O (0.7 ml): generalized erythema, abdominal pain	
6	M	33	SR	Neg	B: neg/W: neg/b: neg	<0.35	S (7 g): urticaria	
7	M	23	AS	Neg	B: neg/W: neg/b: neg	5.11	O (1 ml): AS/S (265 mg): urticaria, angioedema	
8	F	25	Asth	Neg	B: neg/W: neg/b: neg	0.51	O (20 ml): palpebral erythema, abdominal pain/S (965 mg): erythema, abdominal pain	
9	F	4.5	Asth	11 mm	B: 20 mm/W: 7 mm /b: 11.5 mm	44		S: Grade 3
10	F	17	SR	ND	B: neg/W: 2 mm/b: 1 mm	4.3	O (16 ml): negative/S (965 mg): urticaria	
11	M	3	Asth	ND	B: 17.5 mm/W: 4 mm /b: 11.5 mm	76.2		S: Grade 3
12	M	6	Asth	ND	B: 0.5 mm/W: 2 mm /b: 2 mm	9.01	S (7 g): abdominal pain, cough and wheezing	
13	M	36	AS	1.5 mm	B: 5 mm/W: 6.5 mm /b: nd	<0.35	S (7 g): generalized prurit is erythema	S: Grade 1
14	F	3	AD	ND	B: 13 mm/W: 8 mm /b: 11 mm	8.26	S (965 mg): exacerbation of AD	
15	M	13	SR	ND	B: 8.5 mm/W: 17 mm /b: 15.5 mm	13.1		S: Grade 2
16	M	4	Asth	ND	B: 10 mm/W: 8 mm /b: 9.5 mm	40		S: Grade 3
17	M	32	AS	ND	B: 10 mm/W: nd/b: nd	<0.35		O: Grade 3
18	F	10	AO	8 mm	B: 12.5 mm/W: 5.5 mm /b:13.5 mm	16.5	S (965 mg): urticaria, wheezing, and vomiting	
19	M	4	SR	ND	B: 8.5 mm/W: 18 mm /b: 10 mm	4.59	O (6 ml): negative	
20	M	47	AS	ND	B: 9 mm/W: 6 mm /b: 6 mm	ND	S (6 mg): generalized pruritis, erythema on the neck	
21	M	11	Asth	ND	B: 8.5 mm/W: 6 mm /b: 4.5 mm	20.5	S (2 g): asthma	
22	M	22	Asth	3.5 mm	B: 3 mm/W: 4 mm/b: nd	13.2	S (10 g): asthma, erythema	
23	M	7	Asth	ND	B: 13 mm/W: 11 mm /b: 12 mm	>100	S (7 g): abdominal pain, vomiting, conjunctivitis, eczema	S: Grade 2
24	M	4	Vom	ND	B:12.5 mm/W: 14.5 mm /b:7 mm	15.8	S (7 g): negative	
25	M	4	SR	ND	B: 3 mm/W: 12.5 mm /b:15 mm	>100	ND	
26	F	70	AO	ND	B: neg/W: neg/b: neg	<0.35	ND	
27	F	31	SR	Neg	B: neg/W: neg/b: neg	<0.35	ND	
28	F	26	RAS	ND	B: neg/W: neg/B : neg	<0.35	S (965 mg): negative	
29	M	4	SR	ND	B: neg/W: neg/b: neg	<0.35	S (7 g): negative	
30	M	12	AO	ND	B: 3.5 mm/W: 5.5 mm /b: 5.5 mm	0.41	S (7 g): negative	
31	M	21	AO	6.5 mm	B:13.5 mm/W: 15.5 mm /b:14 mm	4.58	ND	
32	M	32	AO, U	ND	B: 7 mm/W: 10 mm /b: 9 mm	<0.35	ND	

RAS, recurrent anaphylactic shock; nf, near fatal; AS, anaphylactic shock; SR, systemic reaction; Asth, asthma; AD, atopic dermatitis; AO, angioedema; Vom, vomiting; LT, labial test; B, brown; W, white; b, black; M, male; F, female; Neg, negative; ND, not detectable; DBPCFC, double-blind placebo-controlled food challenge; IgE, immunoglubulin E.

monospecific rabbit antisesame oleosin (15 or 17 kDa) antiserum (1 : 2000 dilution; 25). Rabbit IgG were detected by antirabbit IgG conjugated with alkaline phosphatase (1/10 000 dilution; Sigma-Aldrich, St Louis, MO, USA) in blocking buffer. After washing, color development was performed by incubating the strips in Tris, NaCl, $MgCl_2$ buffer containing 0.33 mg/ml nitro-blue tetrazolium and 0.165 mg/ml 5-bromo-4-chloro-indolyl phosphate at room temperature in the dark until a sufficient coloring occurred. The reaction was stopped by rinsing the membrane in water.

Results

Patients

Clinical symptoms were of immediate type – anaphylactic shock: seven, recurrent anaphylactic shock: three, systemic reaction: seven, asthma: eight, angioedema: five, and vomiting: one. A single patient had atopic dermatitis. Twenty-seven patients had DBPCFC or labial tests. Fourteen of 32 DBPCFC to seeds were positive and six of 14 to oil. Labial tests to seeds were positive in five of five patients and one of one to oil. Five patients were not challenged according to the severity of the clinical symptoms. Ten of 32 patients (with positive labial test or DBPCFC in seven cases), showed negative SPTs to all sesame extracts and no detectable specific IgE by CAP-FEIA, confirming the difficulty of sesame seed allergy diagnosis.

Specific IgE

Direct ELISAs were performed to assess the level of patient-specific IgE to sesame seed by comparing WS and BS extracts. Whereas ImmunoCAP were found negative in 13 cases, nine patient sera displayed IgE-binding to WS and BS extracts by ELISA. White sesame seed extract was used for the following studies.

IEF immunoblotting. The IEF separation of WS extract followed by immunoblotting showed numerous bands recognized by serum IgE of all patients, even from those with negative CAP-FEIA System (Fig. 1). The isoelectric points of the bands ranged from 4.5 to 8.5.

SDS-PAGE immunoblotting. White sesame extract was separated by SDS-PAGE under nonreducing (Fig. 2) or reducing conditions (Fig. 2). Under nonreducing conditions, allergens were identified migrating between 43 and 67 kDa and between 15 and 17 kDa (Fig. 2). Under reducing conditions, the silver staining revealed that the 15–17 kDa band, observed in the nonreducing conditions, dissociated into a smaller molecule of 9 kDa, corresponding to the 2S albumin large subunit (Fig. 3). However, a doublet located at 15 and 17 kDa was still observed after immunoblotting (Fig. 3), which was presumably oleosin isoforms (Ole).

To prove that oleosins are major allergens, SDS-PAGE immunoblotting of purified oil body fraction (Fig. 4) was performed. The results showed three major bands between 15 and 17 kDa corresponding to oleosins isoforms, as detected by rabbit monospecific anti-15 kDa and 17 kDa oleosin.

Immunoblots showed that 29 over 32 sera have specific IgE to the 15.5 and 17 kDa oleosins. The IgE-binding to both oleosin isoforms was much stronger in case 1 who exhibited a prelethal shock to a mixture of flavoring containing a Mexican sesame oil.

Discussion

The IgE-dependent sensitization to foods may not necessarily coincide with positive prick tests to commercial extracts, because a maximum of diagnostic sensitivity (i.e. 100%) is difficult to achieve. However, the possibility of

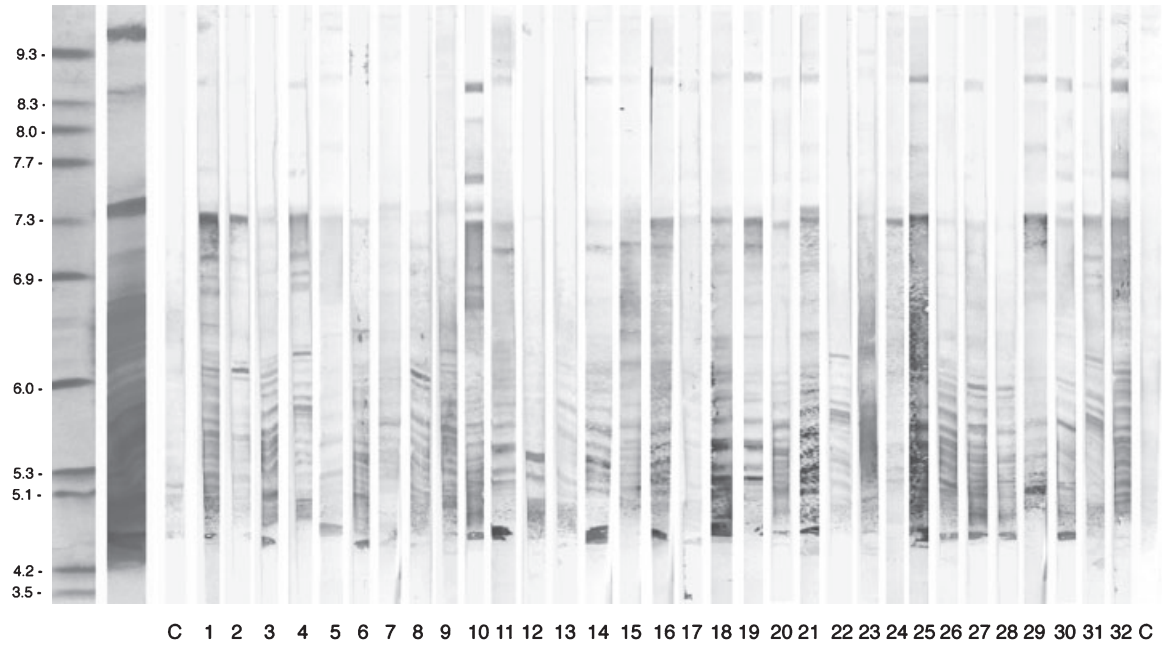

Figure 1. Specificity of immunoglobulin (Ig)E of the 32 patient sera on sesame extract separated by isoelectrofocalisation (IEF) followed by immunoblotting. On the left, isoelectric point markers and sesame extract stained by Coomassie Brilliant Blue. All sera were diluted 1 : 5 in blocking buffer (C: control with blocking buffer).

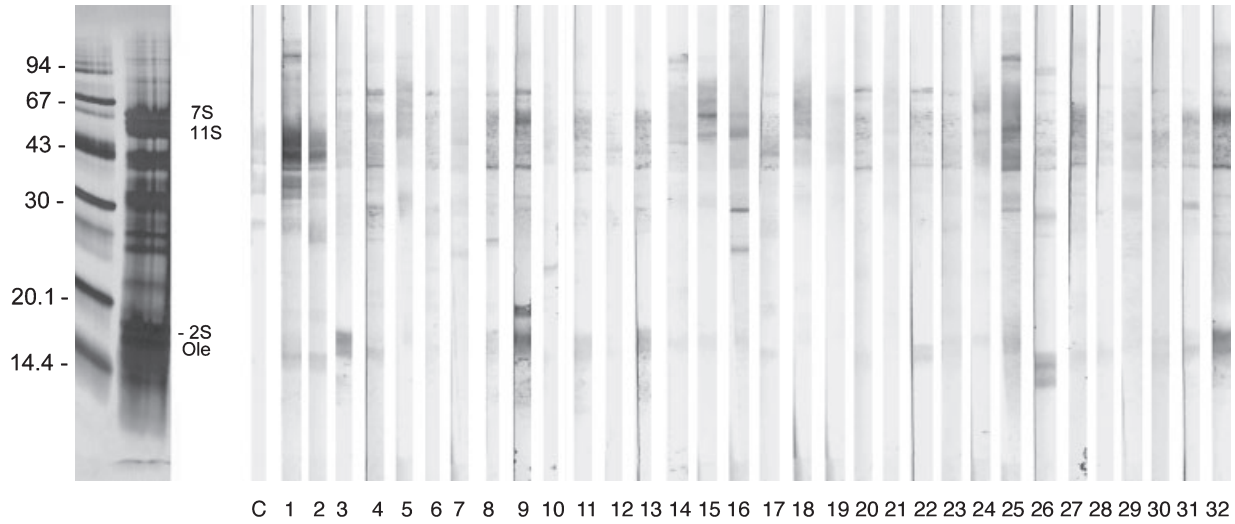

Figure 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of sesame seed extract. Silver-stained gel in nonreducing conditions and specificity of immunoglobulin (Ig)E of the 32 patient sera (1–32). All sera were diluted 1 : 5 in blocking buffer (C: control with blocking buffer).

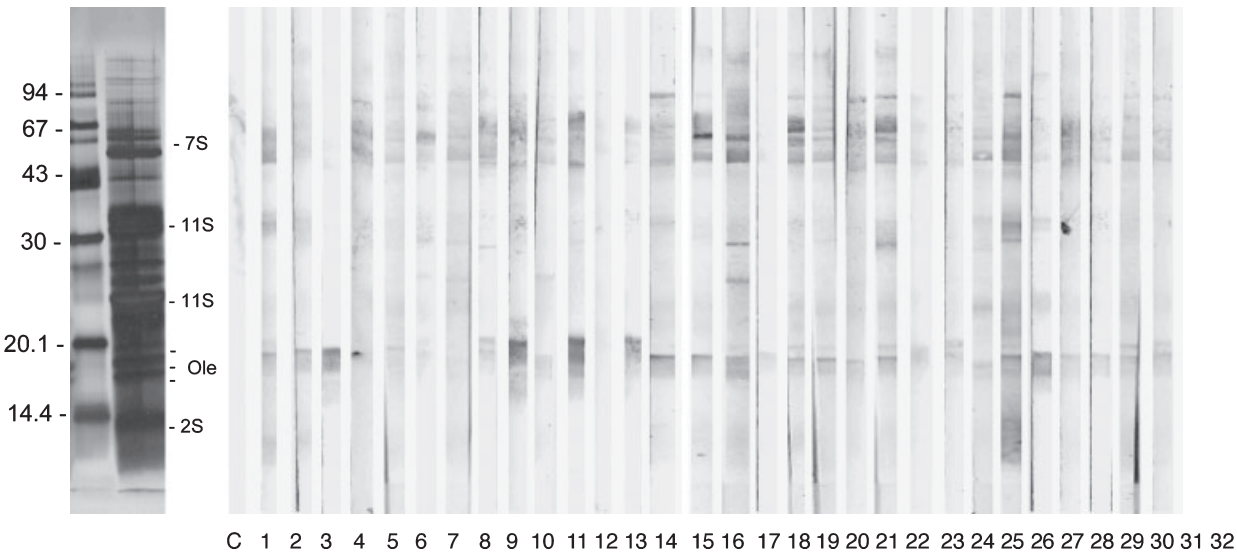

Figure 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of sesame seed extract. Silver-stained gel in reducing conditions and specificity of immunoglobulin (Ig)E of the 32 patient sera (1–32). All sera were diluted 1 : 5 in blocking buffer (C: control with blocking buffer).

falsely negative SPT is often linked to the nature of the food, and is characteristic of aqueous fruit and vegetables (26, 27). Extracts of seeds are currently the most efficient reagents as they are directly related to the concentration of proteins in the seeds. With this in mind, the fact of no evidence of positivity of SPT to three natural varieties of sesame seeds is rather surprising. As sesame seeds are crushed in a saline solution, we raised the hypothesis of the presence of hydrophobic allergens that are insoluble in saline.

Specific IgEs were detected by ELISA and binding of IgE to numerous proteins were demonstrated after immunoblotting of IEF gels. Applying both techniques, the sesame seed proteins were not denatured. Conformational epitopes may be detected, which might otherwise escape recognition by the CAP-FEIA, where the coupling procedure could alter these epitopes.

The comparison of the protein profiles on SDS-PAGE in both reducing and nonreducing conditions revealed several groups of allergens. The first group consisted of

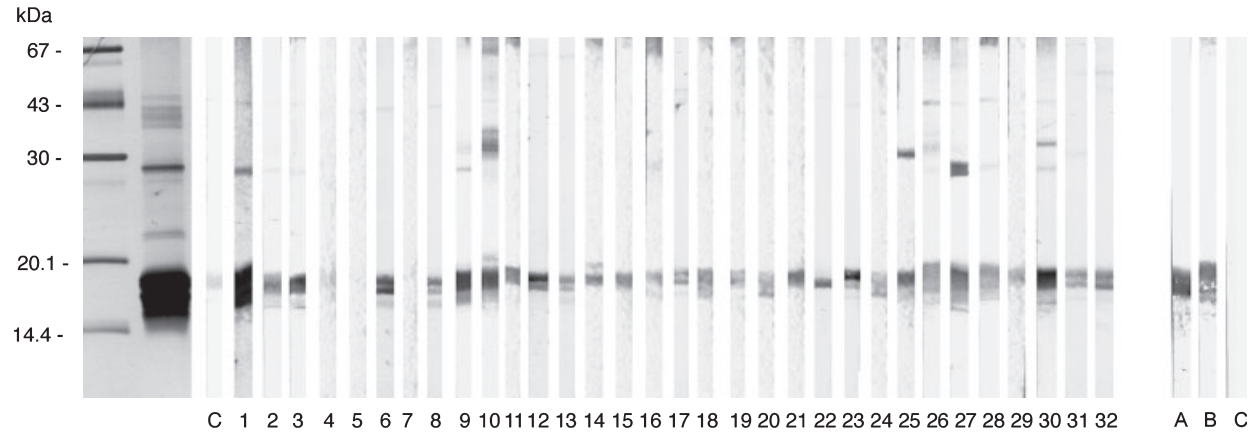

Figure 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting of purified sesame seed oil bodies. Silver-stained gel and immunoblot revealed with immunoglobulin (Ig)E of patient sera (1–32). Lanes: (A) monospecific polyclonal rabbit antibody (Ab) to 15-kDa oleosin; (B) monospecific polyclonal rabbit Ab to 17-kDa oleosin; (C) control with blocking buffer.

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                              1       10        20        30        40        50        60
Q9FUJ9 Oleosin 17kDa          MADRDRPHPHQIQVHPQHPHRYEGGVKSLLPQKGPSTTQILAIITLLPISGTLLCLAGIT
Q9XHP2 Oleosin 15kDa          MAEH-----YGQQQQTRAPHL----------QLQPRAQRVVKAATAVTAGGSLLVLSGLT
                              **::     :  * ::: **           *  * : :::   * :: :*:** *:*:*

                                      70        80        90       100       110       120
Q9FUJ9 Oleosin 17kDa          LVGTLIGLAVATPVFVIFSPVLVPAAILIAGAVTAFLTSGAFGLTGLSSLSWVLNSFRRA
Q9XHP2 Oleosin 15kDa          LAGTVIALTIATPLLVIFSPVLVPAVITIFLLGAGFLASGGFGVAALSVLSWI---YRYL
                              *:**:*:*::***::**********:* *    ::**:**:**:::** ***:   :*

                                     130       140       150         160
Q9FUJ9 Oleosin 17kDa          TGQGPLEYAKRGVQEGTLYVGEKTKQAGEA--IKSTAKEGGREGTART---
Q9XHP2 Oleosin 15kDa          TGKHP--------PGADQLESAKTKLASKAREMKDRAEQFSQQPVAGSQTS
                              **: *          :    : *** *::*  :*: *:: ::: :* :
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Figure 5. Amino acid sequences of the 17 and 15 kDa sesame oleosins, respectively Ses i 4 and Ses i 5. The single-letter amino acid code is used. A dash indicates a gap introduced for the purposes of alignment. '*' means that the residues in the column are identical in both sequences in the alignment; ':' means that conserved substitutions have been observed.

11S globulins that represent 60–70% of the total seed proteins (28). Each of the 11S globulin isoforms consists of an acidic subunit (30–40 kDa) and a basic subunit (20–25 kDa) linked by a disulfide bond (29). Group 2 is the major soluble protein, 2S albumin, constituting approximately 15–25% of the total sesame proteins (30). The two 2S albumin isoforms consist of a small subunit (4 kDa) and a large subunit (9 kDa), linked by a disulfide bond. Under nonreducing conditions, oleosins migrate at the same molecular weight as 2S albumins (15–17 kDa). The third group of two 7S globulin isoforms of 55–60 kDa, represents 1–2% of the total proteins: they have been identified as minor constituents in protein bodies (31). In contrast to 11S globulin and 2S albumin, 7S globulin is a single polypeptide, recognized by nearly all the patient' sera.

Oil bodies are discrete spherical organelles that are also named lipid bodies, or oleosomes and their storage lipids are mainly composed of triacylglycerols (TAGs) in most seeds. The abundant protein referred to as oleosins, which represent 80–90% of total oil body proteins, corresponds to only 1–2% of total seed proteins. Three different oleosins, 17, 15.5 and 15 kDa have been described (32), and 17 and 15 kDa oleosins have been sequenced (Fig. 5). These *non glycosylated* proteins are present at the surface of oil bodies and play a structural role to stabilize the organelles during desiccation of the seed by preventing coalescence of the oil. In this study, we were able to identify oleosins in oil bodies that were recognized by IgEs from all patient sera. The intensity of antibody binding is striking in case 1 (anaphylactic shock after ingestion of sesame oil), leading us to suspect that the majority of oleosins remains residually present in oil and is probably not denatured, as sesame oil is only cold-pressed. The specific risk of sesame oil in allergic responses has been pointed out previously (20, 33). Indeed, patients can react by anaphylactic shock to only a few milliliters of sesame oil (20). Other oils such as peanut or soybean oil do not exhibit such severe reactions, even in highly sensitized subjects allergic to peanuts or to soybeans. Interestingly, the presence of oleosin in peanut oil has been shown and the allergenicity of a recombinant peanut oleosin has been established (34).

In conclusion, we have identified oleosins as new sesame seed major allergens, present in seeds and presumably in oil. The IgEs from all sesame allergic patients studied consistently bound to oleosins. To explain the negativity of SPT to natural sesame, a first hypothesis could be that, in these six patients, the amount of specific IgEs might be predominantly directed to oleosins. The negativity of SPT to natural varieties of seeds could indicate that oleosins are not solubilized in saline, or, alternatively that their epitopes are hindered by the association of oleosins to the TAG, or in the inner part of the molecules, masked by folding of the tertiary structure. If such is the case, the negativity of SPT to natural varieties crushed in saline could imply that these patients have IgEs directed toward epitopes of oleosins buried in the inner part of the molecules. However, the results of ELISA do not support this hypothesis. Neither the level of specific IgE to oil bodies, nor the ratio of IgE to oil bodies/total sesame seed differs in patients with negative or positive PT to sesame seeds (data not shown). This issue cannot be elucidated at present.

Two oleosin (17 and 15 kDa) sequences are known (Fig. 5). According to the IUIS Nomenclature, we submitted these oleosins as Ses i 4 and Ses i 5, respectively. They may characterize severe anaphylaxis without evidence of specific IgEs by the present methods of diagnosis.

Some homology between oleosins of different species has been found for a Chinese spice shiso (*Perilla frutescens*, 75% identity) and for carrot oleosin (64% of identity). Sequence comparison (BLAST) with peanut and soybean oleosins showed lower levels: 56% and 51%. Higher levels of identity can be reached if the sequence is restricted to the central part of oleosins. This domain whose sequence is conserved, is highly hydrophobic and interacts with the lipids. Moreover, this central domain does not present any trypsin cleavage site.

This study supports the obvious need to improve the quality of extracts of sesame for diagnosis. Moreover, it is noteworthy that detection tests for masked sesame allergens are based on vicilins (35). The fact that oleosins are major allergens supports the proposition of oleosins as new markers of masked allergens.

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